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Examining distal humerus morphological variation in Thai individuals using elliptical Fourier analysis

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EXAMINING DISTAL HUMERUS MORPHOLOGICAL VARIATION IN THAI INDIVIDUALS USING ELLIPTICAL FOURIER ANALYSIS

by

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DEDICATION

I would like to dedicate this work to Paul W. Rowell, for always believing in my intelligence, talents, and ambitions.

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ABSTRACT

Sexual dimorphism of the distal humerus has been used for the development of morphometric sex estimation methods in human identification. In particular, visual assessment of the olecranon fossa, trochlear shape, and medial epicondyle angle are variably successful in differentiating females and males in African, Asian, and European groups. However, the influence of other factors on the distal humerus has yet to be fully explored. This study utilizes elliptical Fourier analysis (EFA) to examine the shape of these three features for evidence of sexual dimorphism and the effects of age-at-death, stature, and humeral measurements in 261 modern Thai individuals (f=116; m=145), 20 - 97 years of age. Left humeri were measured, photographed, traced, and analyzed in SHAPE v. 1.3 for EFA. Chi-square, ANOVA, and principal component results indicate sexual dimorphism in the olecranon fossa and trochlear extension shapes, both of which are correlated with epicondylar breadth. Trochlear extension was also found to be correlated with minimum midshaft diameter, vertical head diameter, and stature. The medial epicondyle was not correlated with any of the other factors examined, and age was not correlated with any of the shapes. High rates of intra- and interobserver error were found in the tracings of the three features. While future research should assess methods that better capture the medial epicondyle and improve reliability, features of the

distal humerus are sexually dimorphic and somewhat affected by stature and/or body size.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variances
cm	Centimeters
EB	Epicondylar breadth
EFA	Elliptical Fourier analysis
EFD	Elliptical Fourier descriptors
F	Female
M	Male
MaxMD	Maximum humeral midshaft diameter
MFL	Maximum femur length
MHL	Maximum humeral length
MinMD	Minimum humeral midshaft diameter
mm	Millimeter
PCA	Principal component analysis
VHD	Vertical humeral head diameter

INTRODUCTION

While sexual dimorphism of the distal humerus has been extensively explored (Ammer et al. 2019; Fayls et al. 2005; Rogers 1999, 2009; Tallman and Blanton 2020; Vance et al. 2011), the interactive effects of age, body size, and stature on distal humerus morphology have yet to be studied. It is important to understand how biological and anatomical factors influence sexually dimorphic features, as these factors may impact methodological accuracy. To assess whether distal humerus morphological variation is influenced by sex, age, stature, and/or humeral measurements (i.e., body size), this research utilizes elliptical Fourier analysis (EFA) of olecranon fossa shape, trochlear extension, and medial epicondyle shape.

Distal Humerus Morphology

Features of the distal humerus, including the olecranon fossa shape, trochlear shape, and medial epicondyle angle have been documented to be sexually dimorphic (Rogers 1999, 2009; Fayls et al. 2005; Vance et al. 2011; Tallman 2016; Ammer 2019; Tallman and Blanton 2020). This is attributed to their relationship with the carrying angle of the elbow, which is the angle formed by the deviation of the forearm relative to the long axis of the arm (Rogers 1999). Discussions of the mechanisms of the carrying angle have a long history (Potter 1985; Mall 1905; Steel and Tomlinson 1958; Atkinsen and Elftman 1945; Morrey and Chang 1976; London 1981; Paraskeves et al. 2004), where the orientation of the trochlea, combined with aspects of the ulna, produce a lateral deviation of the forearm (Mall 1905). Sexually dimorphic differences in the carrying angle were first noted by Brocke (1873), but more thoroughly described by Hooton (1946), who claimed that the differences in body proportions between females and males account for the differences in carrying angles. Females, who typically have narrower shoulders and broader hips, have more laterally deviated forearms, and therefore larger carrying angles. The opposite is true for males, who have a smaller carrying angle due to their broad shoulders and narrow hips (Hooton 1946). In addition to the carrying angle being greater in females, Paraskevas et al. (2004) also found that the carrying angle was significantly greater younger individuals (ages 12-18 years) and individuals considered obese.

Morphological analysis of the distal humerus for forensic sex estimation was first proposed by Rogers (1999), who posited that the olecranon fossa shape and depth, trochlear extension, medial epicondyle angle, and trochlear constriction were influential in or the result of the carrying angle. Using skeletal samples from the Grant Skeletal Collection at the University of Toronto (f=10; m=10) and the Documented Collection in the University of New Mexico's Department of Anthropology (f=19; m=20), Rogers (1999) noted that females typically display a constricted and symmetrical trochlea, a deep and oval olecranon fossa, and a raised medial epicondyle, while males typically display a less constricted and extended trochlea, a shallow and triangular olecranon fossa, and a generally flat medial epicondyle (Rogers 1999). When tested on a holdout sample of European American individuals (f=19; m=74) from the William M. Bass Donated Skeletal Collection, the most accurate trait was trochlear constriction (88%), followed by the medial epicondyle angle (86%), olecranon fossa shape and depth (82%), and trochlear extension (69%). Combining the traits with equal weight produced an accuracy of 88.6%, while a combination with greater emphasis on the olecranon fossa in intermediate cases increased accuracy to 94%. Additionally, Rogers (2009) further demonstrated distal humerus sexual dimorphism in juveniles and late adolescents 11-20 years old. Estimations were made by using the “majority rules” approach; if the score was split evenly (i.e., two traits suggesting male, two suggesting female), the olecranon fossa shape and depth was used to make the final estimation, which produced an accuracy rate of 82% for females and 80% for males.

Fayls et al. (2005) conducted a test of Rogers’ (1999) technique on a sample of 167 females and 184 males from the documented skeletal collection at St. Bride’s Church, London. Method accuracy was first tested by using a combination of all the traits proposed by Rogers (1999) followed by a test of each trait individually. Combining the traits produced an accuracy of 76.2% for pooled females and males (Fayls et al. 2005). Traits used individually produced a range of accuracies, with the highest derived from olecranon fossa shape (81.8%), followed by trochlear extension (78.9%), medial epicondyle angle (74.9%), and trochlear constriction (69.2%). Additionally, no significant interobserver differences were found. However, Fayls et al. (2005) found two variances of “intermediate” individuals: individuals who showed an equal number of female and male traits, and those where sex could not be estimated for one or more trait. To accommodate this variation, the authors suggested using each trait individually, rather than basing the estimated sex on the overall morphology of the distal humerus, or introduce more categories (e.g., probable female and probable male). Additionally, they presented line drawings of the trochlea to provide a visual aid for assessing its variation.

Similar to Fayls et al. (2005), Vance et al. (2011) aimed to test the accuracy of features of the distal humerus, but with the application of a composite scoring system on a sample of 420 female and 188 male Black and White South Africans between the ages of 19 and 94 years from the Pretoria Bone Collection at the Department of Anatomy, University of Pretoria and the Raymond A. Dart Collection at the University of Witwatersrand. The authors eliminated trochlear constriction during their analysis due to poor its classificatory accuracy. Rather than assessing overall morphology, the olecranon fossa shape, medial epicondyle angle, and trochlear extension were scored against a five-point scoring system: 1, clearly male; 2, cautiously male; 3, ambiguous; 4, cautiously female; and 5, clearly female. Scores for each feature were combined to produce composite scores of 3-15 (3-8 = male; 9 = indeterminate; and 10-15 = female) (Vance et al. 2011). When used alone, the most accurate trait was the medial epicondyle angle (55% for females and 70% for males). The olecranon fossa was moderately accurate (61% in females and 57% in males), and the least accurate trait was trochlear extension (56% for females and 45% for males). When the traits were used together, this method was 77% accurate in females and 74% accurate in males (Vance et al. 2011).

Tallman and Blanton (2020) assessed the performance of Vance et al.’s (2011) method on a modern Thai sample comprised of 198 females and 418 males from the skeletal collections at Khon Kaen University's Faculty of Medicine, Khon Kaen, and Chiang Mai University's Department of Forensic Osteology, Chiang Mai. Additionally, Tallman and Blanton (2020) developed population-specific binary logistic and probit regression equations and assessed intra- and interobserver error rates. When used alone, trochlear extension produced the highest accuracies (65.5% for females and 85.2% for males), followed by the angle of the medial epicondyle (63.6% for females and 81.4% for males), olecranon fossa shape (53.8% for females and 79.9% for males), and trochlear constriction (70.6% for females and 47.9% for males). Population-adjusted composite scores (3-8 = likely male; 9-15 = likely female) produced accuracies of 63.4% for females and 74.2% for males. The most successful regression equation produced cross-validated accuracies of 91.7% for females, 88.9% for males, and 90.7% pooled, while the least successful regression produced accuracies of 84.4% for females, 65.6% for males, and 76.8% pooled. Similar to Vance et al. (2011), Tallman and Blanton (2020) found that trochlear constriction was not statistically significant in any of the equations, and was consequently removed. Intraobserver error for the study's first author showed fair-to-moderate agreement between the scores of each trait (n=60). Interobserver error rates between the two authors showed less agreement in scores (n=60), with 81.7-86.0% agreeing or differing by one ordinal score. However, the authors agreed on the overall sex estimation in 73.3% of individuals.

To further investigate distal humerus morphological variation, Ammer et al. (2019) utilized an outline shape analysis approach to assess trochlear constriction and olecranon fossa shape. Humeri of 80 females and 71 males from a modern Portuguese sample at the Identified Skeletal Collection at the University of Coimbra, Portugal were photographed in a standardized view. Computer software was then used to digitize landmarks on the photographs, producing an open curve on the trochlea representing trochlear constriction and a closed outline around the shape of the olecranon fossa, each defined by 16 semi-landmarks. The statistical models produced for trochlear constriction did not perform well; however, the olecranon fossa shape produced favorable results, and a second analysis was performed to assess which variables in the shape were the most sexually dimorphic. Two variables were found that account for the triangularity/roundness of the shape and the convexity/concavity of the shape. A linear discriminant analysis model using this method was 95.00% accurate for females and 92.96% accurate for males (Ammer et al. 2019). Using the data from their study, the authors developed a web application that utilizes a shape simulator. This approach uses a combination of statistical shape analysis and geometric morphometrics to produce a method similar to a scoring system, but with a continuous scale (Ammer et al. 2019).

Elliptic Fourier Analysis

Elliptic Fourier Analysis (EFA) utilizes Fourier descriptors, a type of boundary morphometrics, to provide a quantitative analysis of irregular, closed shapes (Tanaka et al. 2000) without sacrificing important details (Nawrocki et al. 2018). This method allows for the quantitative measurement of irregularly shaped skeletal features that are not easily assessed using standard anthropometric methods, while also accounting for size differences (Tanaka et al. 2000). As a result, a greater understanding is achieved regarding how these features vary across populations and in relation to other biological and anatomical factors. EFA has become an increasingly popular method of analysis in the fields of biological and forensic anthropology for studies of frontal sinus morphological variation and identification (Christensen 2004, 2005); cranial vault shape (Maxwell and Ross 2014); orbit morphology (Gore 2009); nasal aperture shape (McDowell 2012); and individualizing characteristics of the clavicle (Stephan et al. 2014), vertebrae (Paolello and Cabo-Perez 2008), and patella (Niespodziewanski et al. 2016).

Moreover, numerous studies have used EFA to explore sexual dimorphism (Tanaka et al. 2000; Schmittbuhl et al. 2002; Lestrel et al. 2005; Gore 2009; Veleminska et al. 2013; Caple et al. 2018). For example, Tanaka et al. (2000) examined sexual dimorphism in the outline of the proximal humerus of Japanese adults and found that that the average outline of female humeri was smaller than the average for males, and sex differences were found in the orientations of the greater and lesser tubercles. A cross-validation test of the proximal humerus produced correct classification accuracies between 92.8% and 100%. Additionally, Schmittbuhl et al. (2002) found a significant amount of sexual dimorphism in the mandibular outline of European adults, where 91.7% of females and 97.1% of males displayed sexually dimorphic differences. However, after normalizing for size, these percentages decreased to 81.2% for females and 84.1% for males. Similarly, Caple et al. (2018) analyzed lateral skull outlines for differences in sexual dimorphism and ancestry. When assessing sex alone, male skull outlines were found to be larger than females. Once size was normalized, differences in shape were found in the mid-facial region, cranial vault, and mandibular angle. Classification accuracies (between 67% and 82%) decreased by 11-13% after the size factor was removed. Therefore, the inclusion of overall shape and size play a role in assessing sexual dimorphism. However, EFA studies generally do not include how other anatomical or biological factors may contribute to the expression of sexually dimorphic features.

While the distal humerus has been shown to be sexually dimorphic and useful for sex estimation in fragmentary or incomplete cases, little is known about the variables that potentially impact the expression of sexual dimorphism. Therefore, the present study uses EFA as a method to examine the olecranon fossa shape, trochlear extension, and the angle of the medial epicondyle (Rogers 1999; Fayls et al. 2005; Vance et al. 2011; Tallman and Blanton 2020) in modern Thai individuals, a population that has been somewhat neglected in forensic anthropological research (Traithepcanapai et al. 2016; Go et al. 2019). Trochlear constriction was not included in this study, as it was not found to be significant by Tallman and Blanton (2020) modern Thai individuals from the same collection. The EFA results were compared with sex, age-at-death, stature, and humeral measurements to assess how they influence the features of the distal humerus.

METHODS

Sample

This study explores distal humerus morphology in Thai individuals from the collection housed at Khon Kaen University's Human Skeletal Research Centre in the Department of Anatomy, Faculty of Medicine. This collection was developed through a body donation program, where donors were only accepted from the Isan region, a geographically isolated region that makes up approximately one third of the area of Thailand. The collection consists of over 745 individuals who died and were macerated between 1979 and 2014 (Techataweewan et al. 2017). Overall, the remains are in good condition, although some have missing cranial and postcranial elements. Information about each individual was collected from government issued identification cards, death certificates, and self-reported answers to questionnaires. Much of the antemortem information is recorded for each individual, including date of birth, age, and sex. In particular, sex is recorded for 216 females and 466 males (collectively 99.6% of the collection). Age is recorded for 683 individuals (91.7%), and stature, weight, and occupation are known for some.

The size of this collection allowed for a random sample of 116 females and 145 males, producing a total study sample of 261 individuals. Only left humeri were used in this study, as Tallman (2016) found that there were no significant differences between left and right sides in the nonmetric distal humerus traits. However, when the left humerus was not available, the right was substituted. As the development of sexually dimorphic traits does not occur until late adolescence (Sheuer and Black 2004), only adult individuals were assessed in this study. Ages ranged from 20 to 97 years of age for females and 20 to 96 years of age for males. Additionally, stature was recorded for only 124 (46.6%) of individuals in the study sample. Consequently, measured stature from the left femur was used in place of recorded stature to maintain methodological consistency (Mhakkanukrauh et al. 2011).

Measurements

Five standard humeral measurements were taken following Langley et al. (2016) and include maximum length, epicondylar breadth, maximum vertical diameter of the head, maximum diameter at midshaft, and minimum diameter at midshaft. Measured stature was obtained using the regression formula for the maximum femoral length developed by Mahakkanukrauh et al. (2011) using a modern Thai sample, which produces high accuracies for females and males.

Photography

All photos were taken with a Panasonic Lumix GX85 Mirrorless Camera with a 12-32 mm lens that had remote control capabilities. The humerus was positioned on a black felt surface with the anterior aspect resting on the table and posterior side up for all images. First, the distal aspect was photographed by securing the camera on a tripod on a table, with the lens directly perpendicular to the table surface and 33 cm from the posterior surface of the humerus (Figure 1). This view allowed for the visualization of olecranon fossa shape and trochlear extension following Rogers (1999). The second view captured the distal-most aspect of the humerus, with the camera secured on a tripod on the floor and the lens set level and parallel to the table surface (Figure 2). Lines were drawn on the black felt to use as a guide for where to place the humeri to maintain a consistent distance from the camera as well as centered in front of the lens. The distance from the distal-most tip of the trochlea and the center of the lens was 23 cm. This view allowed for the visualization of the angle of the medial epicondyle following Rogers (1999).

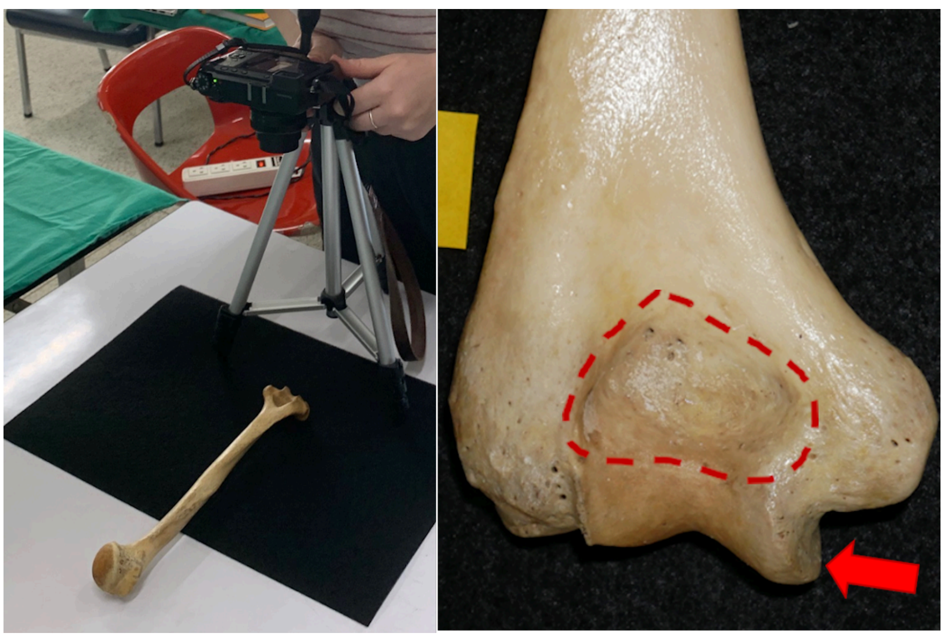

Figure 1. Camera setup for photographs of the posterior aspect of the distal humerus and example image showing olecranon fossa (dashed red outline) and trochlear extension (red arrow).

Figure 2. Camera setup for photographs of the distal aspect of the distal humerus and example image showing angle of the medial epicondyle (dashed red line).

Photograph Tracing

Following photography, the images were uploaded into Adobe Photoshop 2018 CC. The outline of each feature was traced, filled in black, then saved as a separate bitmap image to be compatible with the EFA software. The olecranon fossa was traced following the contours of the fossa using the proximal edge of the trochlea as a guide for the distal border (Figure 3A). When the outline of the olecranon fossa was ambiguous, differences in color from depth were used as a guide. Trochlear extension was first captured by tracing the distal-most outline when viewed from the posterior aspect, including the distal aspects of the medial and lateral condyles, capitulum, and trochlea. An arbitrary cutoff point was designated at the midpoint of the medial epicondyle, producing a straight line across the bone. However, this shape was not refined enough for the SHAPE software to focus on the feature of interest. These shapes where then edited to exclude the unnecessary area. Therefore, two cutoff points were drawn in order to capture the shape of the trochlea alone: a straight, transverse line was drawn level with the distal-most edge of the medial epicondyle; a second straight line was drawn parallel to the shaft, extending from the edge of the trochlea. This produced a shape that included the distal-most extension of the trochlea alone (Figure 3B). The medial epicondyle was first traced using the photographs from the distal view, following the posterior contours of the bone, including the posterior-most aspects of the lateral epicondyle, trochlea, and all of the medial epicondyle. An arbitrary cutoff point was initially designated where the medial epicondyle met the trochlea, and a straight, transverse line was drawn. Like trochlear extension, the tracings of the medial epicondyle included too much area. Therefore, these tracings were also reduced to include only the shape of the medial epicondyle (Figure 3C). This was done by including a second cutoff point at the medial-most edge of the trochlea. The addition of these cutoff points allowed for the EFA to focus directly on the area of the shape that is relevant to this study. As the EFA software assesses all points included in the shape, it was critical to limit the shapes to include only the features with known variation. All other aspects of the bone that were not relevant were therefore excluded from the shapes produced.

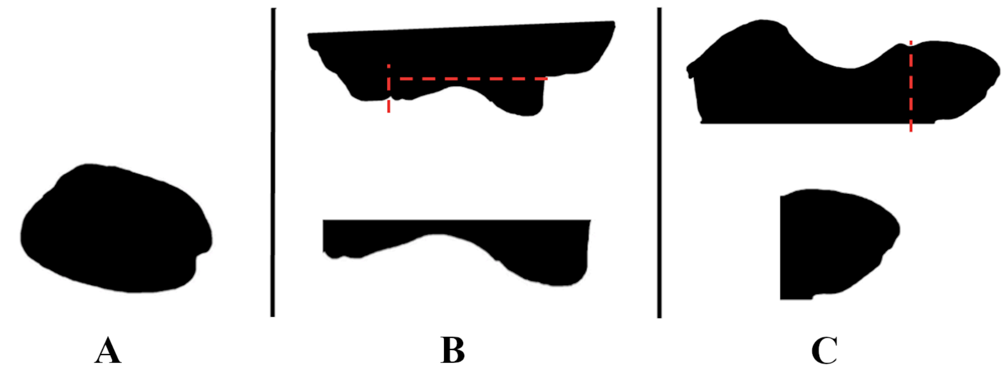

Figure 3. Representative examples of: (A) the final outline for the olecranon fossa; (B) the original (top) and final (bottom) outlines of trochlear extension; and (C) the original (top) and final (bottom) outlines of the medial epicondyle.

EFA Processing

The EFA stage of analysis was completed using SHAPE version 1.3, a computer software package that is designed for the analysis of biological shapes using elliptical Fourier descriptors (EFDs). This package includes multiple programs for processing the image, recording its contours, processing derivations of EFDs, and visualizing shape variations. It is designed to be user friendly, allowing the researcher to run it without needing an in-depth knowledge of the mathematics involved or computer programming (Iwata and Ukai 2002).

The first two steps in EFA were completed using the ChainCoder and Chc2Nef programs from SHAPE. ChainCoder reads the contours of the image to be analyzed and converts the data to chain-code (Iwata 2006), which is essentially a numeric recording of the shape. This is stored as “mile-marker” points that travel along a closed-loop curve, where the time to travel the total distance of the curve is normalized. For this study, the object color was set to dark, as all tracings were black on a white background, and a scale was not included. The bitmap image files were then uploaded and processed using these parameters. The image was converted to grey scale and binarized. Each image was then labeled and a chain-code was produced. Chc2Nef calculates the normalized EFDs (Iwata 2006). The EFDs are the Fourier transformation coefficients (2 coefficients per curve per harmonic) for the two periodic curves formed by plotting the x- and y- coordinates of the chaincode separately. Thus, there are 4 EFD values for each additional harmonic. This study used 20 harmonics to processes each image, and normalization was based on the first harmonic of each contour. This first harmonic is always in the same form: (1, 1, 1, *d*), where *d* is the ratio of the span of the horizontal axis to the span of the vertical axis. This program also standardizes the size and orientation of each contour when the first harmonic is removed from the analysis, allowing for the comparison of shape alone. As the images were processed through this program, they were turned, if necessary, to be closely aligned to their original biological orientation. The normalized EFDs were then saved as Nef files to be used in the statistical analysis. All the shapes were processed together for each feature, resulting in three Nef files. In other words, all female and male olecranon fossa shapes were processed together, resulting in a single Nef file for the olecranon fossa shape.

Statistical Analyses

To assess how the data from the Nef files categorize the shapes, principal component analyses (PCA) were performed using R. PCA can be used to simplify data by reducing the number of dimensions. For this analysis, the data were reduced to three dimensions. The number of dimensions was empirically and pragmatically determined following an exploratory analyses of different choices for the final number of dimensions. Each EFD combination from the samples was then assigned a value along these three dimensions. While the Nef files contained 20 harmonics, only harmonics 2-10 were utilized, as they represented most of the variation between shapes. The results of the PCA were then assessed using a cluster analysis run in R using the "mclust" package, assigning the shape to one of three clusters. These clusters represent groups that share similar attributes across the three dimensions of the PCA, but in way that these attributes appear different in each cluster. The cluster assignments were then compared to the individual's sex using a chi-square analysis, which is commonly used for comparisons between two different categorical variables. Statistically significant differences between the sexes were demonstrated when the *p*-values were less than 0.05. Age, stature, and humeral measurements were each compared with the cluster analysis using analyses of variance (ANOVAs). This test analyzes three or more distinct populations (i.e., the three clusters) to determine if they are statistically different from each other based on an independent factor (i.e., age, stature, humeral measurements). If the results displayed a high F-value, the independent variable was more likely to be related to the variation, if not the actual cause of the variation, as opposed to differences due to chance variation.

The likelihood of the calculated F-value resulting from no differences between the clusters was measured by its *p*-value. If this value was below a significance level of $\alpha = 0.05$, then there were statistically significant differences in the morphological shape displayed when compared with the independent factors.

Intraobserver and Interobserver Error

In order to assess intraobserver error, a random 10% of the sample (n=26) was selected to be photographed and traced again for EFA and measured again by the first author. An additional 10% was selected for reanalysis by a colleague to assess for interobserver error. Two of these individuals were later removed from the analysis during the tracing phase due to an extensive amount of osteoarthritis. Intra- and interobserver variation in photography and tracings were assessed by running the EFA analysis with both the original and secondary tracings. The cluster assignments were then compared between tracings for the same individuals. Differences in measurements for both intra- and interobserver error was assessed using matched-pairs t-tests. A resulting *p*-value greater than the $\alpha = 0.05$ indicates there are no statistically significant differences between measurements.

RESULTS

Results of the chi-square and ANOVA analyses indicate that the olecranon fossa and trochlear extension shapes are correlated with sex, while none of the three features are correlated with age. Most of the metric variables, including stature, were not correlated with the principal component clusters assigned according to the olecranon fossa and medial epicondyle shapes. The opposite was true for the trochlear extension shape, where most of the measurements were correlated to the cluster assignments.

Olecranon Fossa

Cluster analysis results for the olecranon fossa shapes indicate more individuals are likely to be assigned to the third cluster group (53% of females and 71% of males) than the first cluster group (37.9% of females and 24% of males) (Figure 4). The smallest cluster is the second group, where only 8.7% of females and 4.8% of males were assigned. More females were found in clusters 1 and 2 than males. However, more males were found in cluster 3 (Table 1). The chi-square analysis indicates there are statistically significant differences between sexes (p = 0.013) for cluster assignments from the olecranon fossa shapes (Table 2). The results of the ANOVA indicate there are no statistically significant differences in cluster assignment based on the individuals' age (p = 0.859) or stature (p = 0.890) (Table 3). The same is true for all but one of the humeral measurements – epicondylar breadth (p = 0.015) (Table 3).

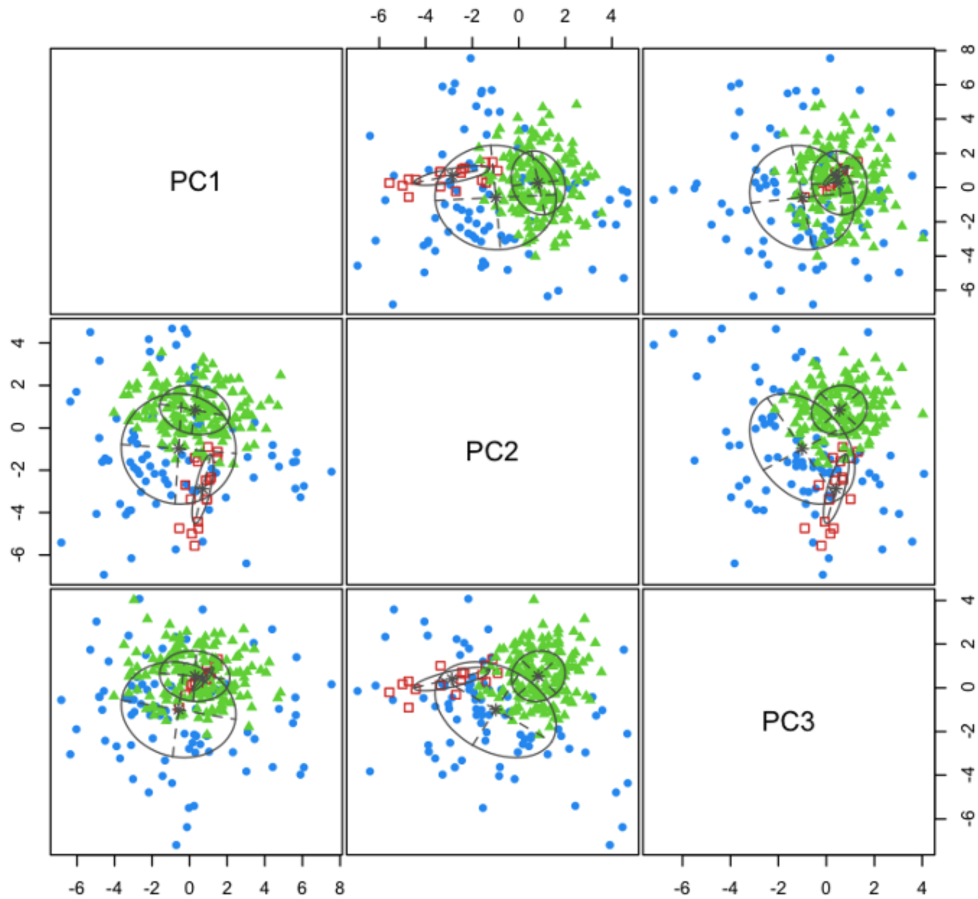

Figure 4. Principal component cluster analysis of the olecranon fossa shapes. Cluster grouping 1 is represented by circles, cluster 2 by squares, and cluster 3 by triangles.

Medial Epicondyle

Most individuals were assigned to the second cluster group (52% of females and 48% of males) based on the medial epicondyle shapes. Assignments to the first and third cluster groupings were fairly even between the groups and sexes, where 25% of females and 24% of males were assigned to the first cluster group and 22% of females and 26% of males were assigned to the third cluster group (Figure 5). Females and males were assigned to each cluster at a fairly even rate (Table 1). The chi-square analysis indicates there are no statistically significant differences between the sexes (p = 0.762) for the cluster assignments from the medial epicondyle shapes (Table 2). The results of the ANOVA analyses revealed that there was no correlation between the other factors assessed in this study, including age, humeral measurements, and stature and the cluster assignments (Table 3).

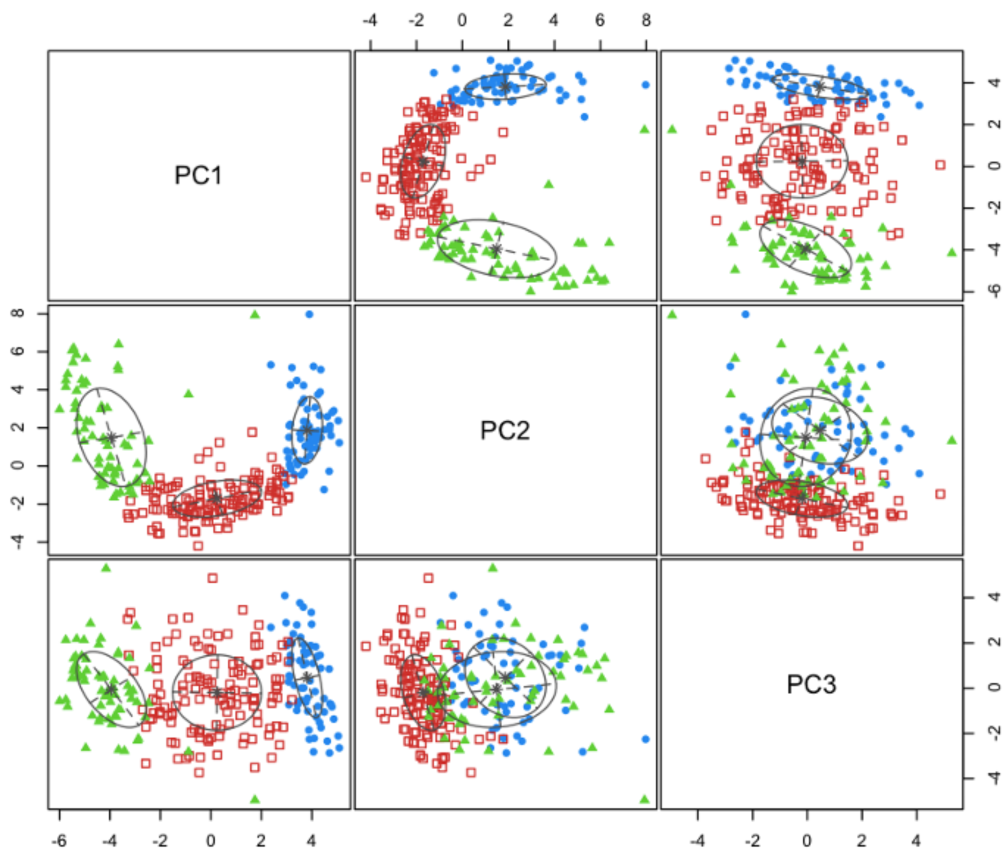

Figure 5. Principal component cluster analysis of the medial epicondyle shapes. Cluster grouping 1 is represented by circles, cluster 2 by squares, and cluster 3 by triangles.

Trochlear Extension

Most females were assigned to the first cluster grouping (50.9%) based on the trochlear extension shape, followed by the second (34.5%) and third (14.7%) cluster groupings. Males were more commonly assigned to the second cluster grouping (54.4%), followed by the third (33.8%) and first (11.7%) cluster groupings (Figure 6). More females were assigned to the first cluster group than males. The opposite is true for the second and third cluster groups (Table 1). The chi-square analysis indicates that there are statistically significant differences between sexes ($p < 0.0001$) for the cluster groupings, more so than the olecranon fossa (Table 2). The ANOVA results indicate there is no statistically significant difference between age groups ($p = 0.530$); however, there were statistically significant differences found for measured stature ($p < 0.0001$) (Table 3). Of the humeral measurements, only two were found to not be correlated with cluster assignments: maximum humeral length ($p = 0.112$) and maximum midshaft diameter ($p = 0.130$). Epicondylar breadth ($p < 0.0001$), minimum midshaft diameter ($p < 0.0001$), and vertical head diameter ($p < 0.0001$) are significantly correlated with assigned cluster groupings (Table 3).

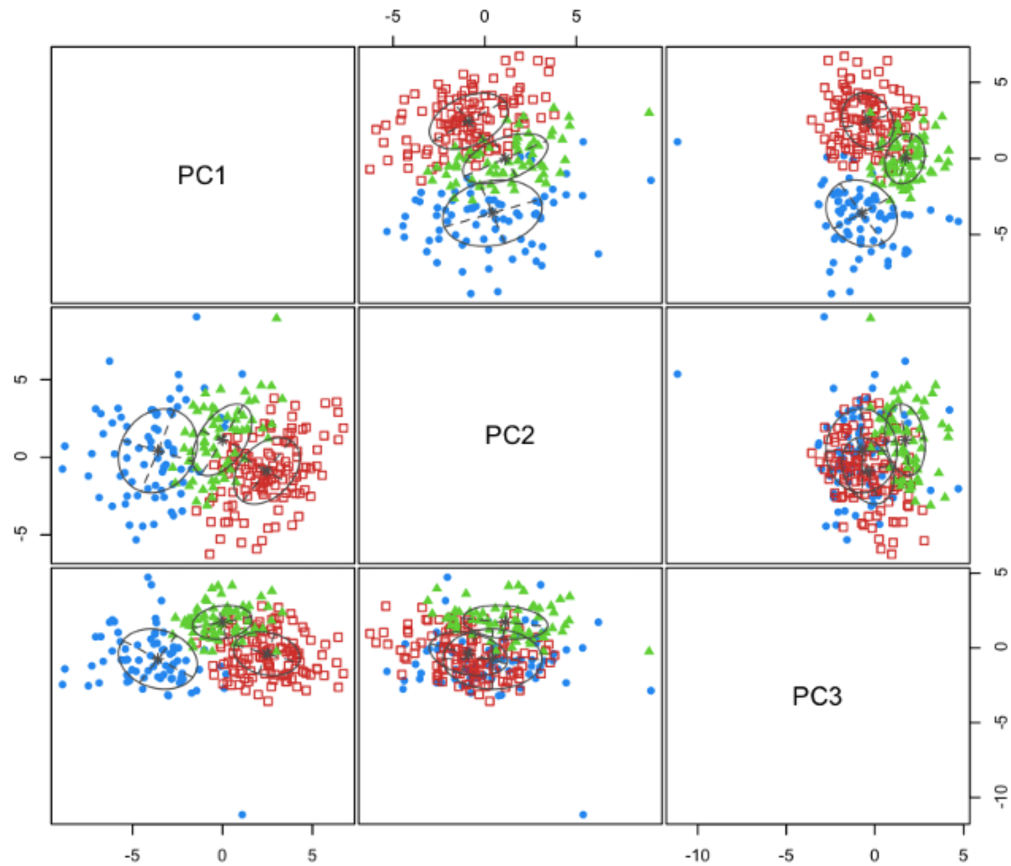

Figure 6. Principal component cluster analysis of the trochlear extension shapes. Cluster grouping 1 is represented by circles, cluster 2 by squares, and cluster 3 by triangles.

Table 1. Distribution of females and males for cluster analyses of the olecranon fossa, medial epicondyle, and trochlear extension.

Shape	Sex	Cluster 1 Number of Individuals (%)	Cluster 2 Number of Individuals (%)	Cluster 3 Number of Individuals (%)
Olecranon Fossa	F	44 (55.7)	10 (58.9)	62 (37.6)
	M	35 (44.3)	7 (41.2)	103 (62.4)
Medial Epicondyle	F	29 (44.6)	60 (45.5)	22 (45.8)
	M	36 (55.4)	72 (54.5)	26 (54.2)
Trochlear Extension	F	59 (77.6)	40 (33.6)	17 (25.8)
	M	17 (22.4)	79 (66.4)	49 (74.2)

Table 2. Chi-square analysis results for sex when compared with cluster analysis results.

Shape	X^2	dF	*p*-value
Olecranon Fossa	8.6269	2	0.01339
Medial Epicondyle	0.5445	2	0.7617
Trochlear Extension	46.065	2	9.94E-11

Table 3. ANOVA results for age, estimated stature, and humeral measurements when compared with cluster analysis results.

Shape	Variables	dF	Sum sq.	Mean sq.	F-value	pr(>F)
Olecranon Fossa	Age	2	78	39.071	39.071	0.8594
	Estimated stature	2	16.7	8.74	8.74	0.8903
	Maximum Length	2	213	106.5	0.3171	0.7285
	Epicondylar Breadth	2	203.2	101.578	4.2576	0.01516
	Max. midshaft diameter	2	180	90.036	0.5029	0.6054
	Min. midshaft diameter	2	22.44	11.222	2.9485	0.5421
	Vertical head diameter	2	10.1	5.067	0.302	0.739
Medial Epicondyle	Age	2	20	9.951	0.038	0.9627
	Estimated stature	2	61.5	30.753	0.4231	0.6555
	Maximum Length	2	117	58.45	0.1743	0.8402
	Epicondylar Breadth	2	123	61.512	2.5557	0.0796
	Max. midshaft diameter	2	126	63.057	0.3532	0.7028
	Min. midshaft diameter	2	0.8	0.4019	0.1044	0.9009
	Vertical head diameter	2	21.8	10.898	0.654	0.5208
Trochlear extension	Age	2	327	163.62	0.6374	0.5295
	Estimated stature	2	2075.4	1037.7	16.233	2.52E-07
	Maximum Length	2	1900.7	950.35	7.9694	0.1115
	Epicondylar Breadth	2	822.8	411.42	19.536	1.30E-08
	Max. midshaft diameter	2	726	363.24	2.0539	0.1304
	Min. midshaft diameter	2	119.3	59.65	17.443	7.942e-08
	Vertical head diameter	2	383	191.49	12.491	6.679e-06

Intraobserver Error

The trait with the lowest intraobserver error rate is the medial epicondyle, where 30.8% of the secondary shapes were assigned different cluster groupings compared to the original shape analysis. Differences in cluster assignments between the original and secondary shapes occurred for 42.3% of the olecranon fossa shapes and 46.2% of the trochlear extension shapes (Table 4). Results of the matched pairs t-tests indicate that there are no statistically significant differences in the first and second measurements of the epicondylar breadth ($p = 0.340$), maximum length ($p = 0.589$), maximum midshaft diameter ($p = 0.17$), vertical head diameter ($p = 0.746$), or maximum femoral length ($p = 0.488$) (Table 5). The test suggests differences in the minimum midshaft diameter ($p = 0.047$). However, first and second measurements do not differ by more than 2 mm, with the exception of one individual.

Table 4. Intraobserver class assignments for the shapes of the olecranon fossa, medial epicondyle, and trochlear extension.

Shape	Cluster Assignment	Tracing 1 (number of individuals)	Tracing 2 (number of individuals)
Olecranon Fossa	1	16	12
	2	4	5
	3	6	9
Medial Epicondyle	1	12	7
	2	8	11
	3	6	8
Trochlear Extension	1	10	13
	2	6	9
	3	10	4

Table 5. Intraobserver error results from matched paired t-tests for epicondylar breadth (EB), maximum humeral length (MHL), maximum midshaft diameter (MaxMD), minimum midshaft diameter (MinMD), vertical head diameter (VHD), and maximum femoral length (MFL; used for stature estimation).

Measurement	*t*	dF	*p*-value	Mean of diff.
EB	-0.9723	25	0.3402	-0.2308
MHL	-0.5473	25	0.5891	-0.6538
MaxMD	1.4131	25	0.17	0.1923
MinMD	2.0868	25	0.0473	0.3462
VHD	-0.32756	25	0.746	-0.0769
MFL	-0.7051	25	0.5891	-0.6538

Interobserver Error

Interobserver error rates were generally higher, where 33.3% of the medial epicondyle shapes and 37.5% of the trochlear extension shapes were assigned different shape classes between observers. The olecranon fossa shape shows a high interobserver error rate, with 58.3% of shapes assigned to different classes between observers (Table 6). Results of the matched pairs t-tests indicate agreement between observers in measurements of the epicondylar breadth (p = 0.747), maximum length (p = 0.948), maximum midshaft diameter (p = 0.257), and vertical head diameter (p = 0.256) (Table 7). The test suggests differences in the minimum midshaft diameter (p = 0.032). However, none of the measurements differ by more than 2 mm between observers. Results also indicate differences in maximum femoral length (p = 0.009), despite only one instance of the difference in measurements between observers exceeding 2 mm.

Table 6. Interobserver class assignments for the shapes of the olecranon fossa, medial epicondyle, and trochlear extension.

Shape	Cluster Assignment	Observer 1 (number of individuals)	Observer 2 (number of individuals)
Olecranon Fossa	1	10	2
	2	7	6
	3	7	16
Medial Epicondyle	1	14	9
	2	3	2
	3	7	13
Trochlear Extension	1	10	13
	2	5	3
	3	9	8

Table 7. Interobserver error results from matched pairs t-tests for epicondylar breadth (EB), maximum humeral length (MHL), maximum midshaft diameter (MaxMD), minimum midshaft diameter (MinMD), vertical head diameter (VHD), and maximum femoral length (MFL; used for stature estimation).

Measurement	*t*	dF	*p*-value	Mean of diff.
EB	-0.3271	23	0.7466	-0.8333
MHL	0.0656	23	0.9484	0.0417
MaxMD	-1.1632	23	0.9383	-0.1667
MinMD	-2.2895	23	0.03156	-0.2917
VHD	-1.155	23	0.2559	-0.2083
MFL	2.8868	23	0.0088	0.9091

DISCUSSION

A research gap exists in the exploration of biological and anatomical factors that affect shape variation of the distal humerus features. This study analyzed distal humerus morphological variation, allowing for a more in-depth understanding of the differences between females and males, and the factors that possibly impact distal humerus morphology. As an increasingly popular method of analysis in the field of biological anthropology, EFA can be an important tool for capturing minute characteristics of bone morphology that can be easily overlooked by the human eye or traditional metrics. Additionally, quantitative methods of analysis, such as EFA, are potentially more objective and therefore more reliable than visual analysis alone; however, the shape outline tracings introduce considerable subjectivity as indicated by high intra- and interobserver error rates. Using EFA and cluster analysis as a means of analyzing the features of the distal humerus allows them to be categorized using shape alone, separate from their assumed sexual dimorphism. While the clusters are arbitrarily based on shape, they offer insight into these shapes and the factors from which they are influenced. This method of analysis allowed for a direct comparison of the differences in shape with sex, age, stature, and humeral measurements.

The results of the present study agree with those previous (Fayls et al. 2005; Rogers 1999; Vance et al. 2011; Tallman and Blanton 2020) in that the shapes of the olecranon fossa and trochlear extension are sexually dimorphic traits of the distal humerus. Conversely, the use of EFA to quantify the shape of the medial epicondyle did not result in sexually dimorphic differences. This is likely due to how this feature was analyzed as an enclosed shape, rather than the angle at which the medial epicondyle projects from the trochlea, as defined by the distal humerus sex estimation methods (Rogers 1999; Vance et al. 2011; Tallman and Blanton 2020). These studies found that the medial epicondyle produced accuracy rates of 55-89% when used alone for sex estimation, indicating this feature is sexually dimorphic. To assess the true nature of the sexual dimorphism of this feature, the shape will need to be redefined in future studies to better account for the angle at which the medial epicondyle meets the trochlea. However, this may concurrently mask significant data by introducing shape features unrelated to medial epicondyle angle that the EFA methodology will attempt to group.

While Tallman and Blanton (2020) found that age was not significant when added to their regression equations, an analysis of the effects of age on the individual traits revealed a small effect on the medial epicondyle for the male age groups. Individuals in the 60-69, 70-79, and 80-96 age cohorts were more likely to have a medial epicondyle score lower (i.e., more anteriorly positioned) than those in the 30-39 age cohort. Additionally, individuals in the 60-69 and 80-96 age cohorts were also likely to have a lower medial epicondyle score than those in the 40-49 age cohort (Tallman and Blanton 2020). The present study found that age was not correlated with any of the three shapes when analyzed with EFA. Although the results from Tallman and Blanton (2020) show that age possibly influences the morphology of the medial epicondyle, it is only statistically significant for a subgroup and the age affects did not impact the multivariate prediction of sex. Moreover, it is possible that the difference in assessing this trait as an enclosed shape versus an angle relative to the trochlea likely influenced the results found in the present study. However, it is also possible that the trait is not severely affected by age.

Stature was found to be correlated with trochlear extension only. Males typically display a more extended trochlea, and are, on average, taller than females. Thus, it is logical to suggest that a higher stature is associated with a more extended trochlea. The femur was chosen for measured stature, as it produced the greatest accuracies for a modern Thai population (Mhakkanukrauh et al. 2011) and contributes significantly to stature. Interestingly though, the maximum humeral length, a measurement also used for stature estimation (Mhakkanukrauh et al. 2011), was not found to be correlated with trochlear extension, despite its contributions to the measurement. It is likely that differences in body proportions between the arm and thigh could be the cause of the dissimilarities in correlation.

While all of the humeral measurements can be representative of body size, wherein greater body size is associated with larger measurements, only a few were found to be correlated with trochlear extension, including vertical head diameter and minimum midshaft diameter.

As both the trochlea and humeral head are joint surfaces, is logical that changes in the length of one could be reflected in the other - as the humeral head increases in diameter, the trochlea becomes more extended. However, it is interesting that the minimum midshaft diameter is also correlated with the trochlear extension shapes while the maximum midshaft diameter is not. While this could be due a difference in the proportions and shape of the humeral shaft, more research is needed to understand why only one of the midshaft measurements is associated with trochlear extension.

Epicondylar breadth was found to be correlated with both the shapes of the olecranon fossa and trochlear extension. Measurement ranges are consistent between the three clusters for both shapes, making it difficult to determine which aspects of the shapes are associated with epicondylar breadth. Correlation between trochlear extension and epicondylar breadth can also be the result of an overall increase in the size of the bone, much like the relationship between trochlear extension and stature. Where males display a greater maximum humeral length and more distally extended trochlea, they can also show wider epicondylar breadths (Patterson and Tallman 2019). It is also possible that increases in both the length and width of the bone can be influential on the shape of the olecranon fossa. In particular, a wider epicondylar breadth may cause the distal-most aspect of the olecranon fossa to stretch, forming the characteristic triangular shape associated with males. Conversely, a narrower epicondylar breadth would not cause this affect, leaving the olecranon fossa to be the characteristic oval shape associated with females. However, the role the olecranon process of the ulna and its contributions to the olecranon fossa morphology are unknown. Interestingly, epicondylar breadth is not correlated with the medial epicondyle shape, despite its contributions to the measurement. Although males typically express a broader epicondylar breadth, the length at which the medial epicondyle alone projects should not be considered when performing nonmetric sex estimation methods.

Unlike the results of the present study, other EFA studies have found very small amounts of intra- and interobserver error (Caple et al. 2018; McDowell et al. 2012). In a study of ancestry and sex from lateral skull photographs, Caple et al. (2018) found few differences between intra- and interobserver photographs (mean differences being 0.01% and 2.45%, respectively). McDowell et al. (2012) also found insignificant differences between tracings of nasal aperture shape ($p = 0.234$). Meanwhile, intra- and interobserver error rates in the present study are high for all of the shapes, where at least 30% showed differences in cluster assignment between the first and second shape analyses. Given that the distal humerus is also responsible for movement and muscle attachments, it is more morphologically complex than other areas of the skeleton typically analyzed with EFA, such as the nasal aperture, eye orbit, or lateral skull outlines. The olecranon fossa, trochlea, and medial epicondyle are also in close proximity to one another, where aspects of one feature are likely influential on the others (Tallman and Blanton 2020), ultimately leading to variations in the interpretation of shapes during the tracing process. Although measures were taken to ensure consistent photography setup, even the smallest change in camera or bone placement could potentially affect the angle at which the photo was taken. This would produce an image from a slightly different perspective as well as differences in the traced shape, and introduces an increased amount of subjectivity into how these shapes are captured. Therefore, while EFA can be used to detect minute differences in shapes between individuals, the subjective perceptions of these shapes by the observer during the tracing process also plays a significant role.

The olecranon fossa shape showed the highest amounts of error, particularly between observers. While seemingly the most straightforward due to its enclosed shape with no designated cutoff points, the exact perimeter of the olecranon fossa was sometimes difficult to determine, leaving the observer to estimate the outline of the shape. Namely, the olecranon fossa lacks distinct margins, but instead has margins that are rounded and often gently curved. Tallman and Blanton (2020) also found relatively high interobserver error rates when scoring the olecranon fossa shape following Vance et al. (2011). In particular, Tallman and Blanton (2020) found differences in shape distinguishing between the an “oval” and “triangular” descriptions to be subtle. Furthermore, Nawrocki et al. (2018) attribute high rates of intra- and interobserver errors to be the result of random and unexplainable variability in the shapes of skeletal features. Thus, future research should explore the reliability of feature tracings for morphologically complex areas of the skeleton.

In addition to the high level of intra- and interobserver error, the defined shape for each trait, including cutoff points, should be considered when examining the reliability of these results. Because the EFA software assesses the uploaded shape as a whole, it is possible that other features of the shape are factored into analyses. This is less problematic for the shape of the olecranon fossa, where the shape itself is an enclosed contour. However, the assigned cutoff points applied to the medial epicondyle and trochlear extension shapes are arbitrary and established to include only the areas of interest as part of the nonmetric distal humerus sex estimation methods. To assess the influence of these cutoff points, future research on this topic should conduct analyses including multiple shapes with a variety of cutoff points for each shape.

Other studies using EFA highlight how additional factors can also be influential in skeletal morphology. In their study of the outline of the proximal humerus of Japanese adults using EFA, Tanaka et al. (2000) found sex differences in shape when controlled for size. A stepwise discriminant function analysis produced classification accuracies between 86.1 and 100%. Additionally, they found differences in the placement of the greater and lesser tubercles, where males were found to have more postero-medially located greater tubercles and more anteriorly located lesser tubercles, compared to females. This was suggested to be due to the attachment and effects of the rotator cuff muscles. Tanaka et al. (2000) contend that these effects are primarily influenced by environmental factors, rather than genetics. Differences in activity, or other environmental factors, between females and males are potential causes for the differences in tubercular placement. Thus, further research is needed to address how the morphology of the distal humerus is influenced by muscles (e.g., pronator teres, flexor carpi radialis, flexor carpi unlaris, flexor digitorum superficalus, and palmaris longus) and ligaments (e.g., ulnar collateral ligament) attached to the medial epicondyle. Investigation into and understanding how the movement of these muscles changes the morphology can help to strengthen the distal humerus sex estimation method. For example, it is unknown whether the angle of the medial epicondyle is influenced by musculature in addition to the carrying angle. However, Tallman and Blanton (2020) found that younger Thai males display more posteriorly oriented medial epicondyles compared to older males, suggesting that the flexors subtly pull the medial epicondyle anteriorly over time and with use. Thus, it is likely that musculature influences the angle of the medial epicondyle in some individuals.

Schmittbuhl et al. (2002) observed the sexual dimorphism of the lateral mandible, both with and without the effects of size, using uniquely defined Fourier descriptors. They found high classification rates (91.7% for females and 97.1% for males) when analyzing the mandibular outlines without adjustments made for size. After normalizing the outlines for size, accuracy dropped (81.9% for females and 84.1% for males), demonstrating that the inclusion of size can improve accuracy considerably. Similarly, addressing the effects of size using the EFA method can further improve distal humerus sex estimation method. Whereas much of the sexual dimorphism present in the postcranial skeleton is due to differences in size between females and males, particularly in the humerus (Frutos 2005; Kranioti and Michalodimitrakis 2009; İşcan et al. 1998; Lee

2014; Boldsen et al. 2015; Patterson and Tallman 2019), which no doubt influence the shapes of the olecranon fossa, medial epicondyle, and trochlear extension.

While there are likely many factors that contribute to the variation of the morphology of the shapes analyzed in this study, it is unknown how significant of a role they play. Gore (2009) assessed the sexual dimorphism of the orbital cavity, as well as the effects of ancestry and continent of origin, using EFA. When controlled for size, the results showed that the first five principal component (PC) factors accounted for most of the variation in shape, although the first (PC1) was not affected by any of the other factors in the study; however, only a small amount of the variance in shape is explained. For example, PC1 was responsible for the differences in the height of the orbital cavity and is influenced by ancestry and continent of origin, but the variables were only responsible for 2.6% of the variation. Consequently, 97.4% of the variation in this shape is unaccounted for. While we can account for some of the variation seen in the olecranon fossa, medial epicondyle, and trochlear extension, it is unrealistic to assume we will understand it in its entirety. The skeleton is a dynamic system that constantly adapts to the needs of the individual's body. As the skeleton changes throughout an individual's lifetime, the state at which we observe a skeleton may not be completely reflective of the individual's entire life.

Comparison of the distal humeral morphology with other aspects of the biological profile allows anthropologists to better understand how these features are shaped through interacting human biological variables. The lack of correlation with age and minimal correlation with stature and other humeral measurements, combined with evidence of sexual dimorphism based on shape alone, suggests that the olecranon fossa and trochlear extension are indeed suitable for use in sex estimation methods. More research eliminating the image tracing issues is needed to conclude the same for the medial epicondyle when using EFA; however, previous research has proven its utility in nonmetric sex estimation (Vance et al. 2011, Tallman and Blanton 2020, Rogers 1999, Falys et al. 2005). The measurements that were found to be associated with the morphology of the distal humerus are also correlated with sexual dimorphism, where males typically display longer and wider proximal and distal humeri compared to females. Therefore, these variables themselves can be considered sexually dimorphic, further enhancing the sexual dimorphism displayed by these features. If the distal morphological features are not severely influenced by factors other than sex or sexually dimorphic measurements (e.g., age), then sex estimation methods using these features can be used without the need to account for this information, which may not always be available. This also makes adapting the method for a wider variety of population groups an easier task, as only population differences in sexual dimorphism will need to be assessed to adapt the method.

While it is important for these comparisons to be made on a variety of populations, there is a distinct lack of research on Thai individuals. Following the 2004 tsunami, which resulted in the death of more than 5,300 Thai individuals, there has been a growing need for research on biological profiling methods to identify unknown Asian individuals (Traithepcanapai et al. 2016; Go et al. 2019). Sex estimation is a major component of the biological profile as it can dramatically reduce the pool of potential persons, and sex contributes to age and stature estimations. A more comprehensive understanding of sexual dimorphism will allow for increasing the accuracies of existing sex estimation methods and in the development of anatomically informed methods in the future.

CONCLUSION

The purpose of this study was to explore the morphology of the distal humerus and its interactions with sex, age, stature, and humeral measurements using EFA. EFA allows for a complete analysis of the shapes of the olecranon fossa, medial epicondyle, and trochlear extension, including details that can be overlooked when performing nonmetric assessment alone. The results show that the shapes of the olecranon fossa and trochlear extension are significantly correlated with sex. These shapes were also found to be correlated with epicondylar breadth. Additionally, trochlear extension was found to be significantly correlated with measured stature, the minimum midshaft diameter, and vertical head diameter. These results agree with those of previous studies addressing the sexual dimorphism of the distal humerus and its use as a sex estimation method (Fayls et al. 2005; Rogers 1999; Vance et al. 2011; Tallman and Blanton 2020). The medial epicondyle was not found to be correlated with any of the factors addressed in this study. However, additional study is needed to determine if the lack of correlations stems from the chosen tracing method and how the medial epicondyle was represented in its shape. Age was not found to be correlated with any of the three shapes. High intra- and interobserver error rates for the EFA and cluster analysis (30.8-46.2% and 33.3-583%, respectively) were found, and likely result from differences that occurred in the tracing stage of analysis. Humeral measurements showed very little intra- and interobserver error. In addition to investigating the medial epicondyle further, the influences of other factors, including tracing methods, size normalization, and muscle attachments, are needed to provide a more comprehensive understanding of sexual dimorphism in the distal humerus. While there is still much to discover about the morphology of the distal humerus, the results of this study demonstrate that stature and body size somewhat influence distal humerus sexual dimorphism and anthropologists can incorporate this knowledge to improve sex estimation methods.

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